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ORIGINAL ARTICLE

Characterization of nineteen antimony(III) complexes as potent inhibitors of photosystem II, carbonic anhydrase, and glutathione reductase

Mehmet Sayım Karacan[1] · Margarita V. Rodionova[2] · Turgay Tunç[3] · Kübra Begüm Venedik[1] · Serhat Mamaş[1] · Alexandr V. Shitov[4] · Sergei K. Zharmukhamedov[4] · Vyacheslav V. Klimov[4] · Nurcan Karacan[1] · Suleyman I. Allakhverdiev[2,4,5]

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Abstract Nineteen antimony(III) complexes were obtained and examined as possible herbicides. Six of these were synthesized for the first time, and their structures were identified using elemental analyses, ^{1}H-NMR, ^{13}C-NMR, FTIR, LCMS, magnetic susceptibility, and conductivity measurement techniques. For the nineteen examined antimony(III) complexes their most-stable forms were determined by DFT/B3LYP/LanL2DZ calculation method. These compounds were examined for effects on photosynthetic electron transfer and carbonic anhydrase activity of photosystem II, and glutathione reductase from chloroplast as well were investigated. Our results indicated that all antimony(III) complexes inhibited glutathione reductase activity of chloroplast. A number of these also exhibited good inhibitory efficiency of the photosynthetic and carbonic anhydrase activity of Photosystem II.

Keywords Photosystem II (PSII) · Carbonic anhydrase · Glutathione reductase · Antimony(III) complexes · Inhibitors

Introduction

One of the methods to study enzyme function is to use specific inhibitors. However, an application of new chemical agents acting as inhibitors may allow getting more sophisticated information about enzymes and enrich our knowledge. Searching among existing chemicals, or design new ones based on previously obtained data, synthesis and screening these chemicals as potent inhibitors of enzymes is vitally important for organism. This work is essential first step for creation of the plants defense means and herbicides as well as possible preparation for the human and animal medicine.

Weeds are still a limiting factor in crop production all over the world. Weed management largely depends on herbicides because of their high-level capability and relatively low cost compared with other weed-control technologies (Duke and Dayan 2012). Design of new high effective herbicides is especially important taking into account the fast increase of Earth population. Some of currently used herbicides are enzyme inhibitors, for example, those of acetyl CoA carboxylase (ACCase), acetolactate and acetohydroxyacid synthases, glutamine synthase, dihydropteroate synthase, as well as Photosystem I (PSI) and Photosystem II (PSII). The first major herbicide

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✉ Mehmet Sayım Karacan
mkaracan@gazi.edu.tr

✉ Nurcan Karacan
nkaracan@gazi.edu.tr

✉ Suleyman I. Allakhverdiev
suleyman.allakhverdiev@gmail.com

[1] Department of Chemistry, Science Faculty, Gazi University, Teknikokullar, Ankara, Turkey

[2] Controlled Photobiosynthesis Laboratory, Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, Moscow, Russia 127276

[3] Department of Chemistry and Process Engineering, Engineering and Architecture Faculty, Ahi Evran University, Kırşehir, Turkey

[4] Institute of Basic Biological Problems, Russian Academy of Sciences, Institutskaya Street 2, Pushchino, Moscow Region, Russia 142290

[5] Department of Plant Physiology, Faculty of Biology, M.V. Lomonosov Moscow State University, Leninskie Gory 1-12, Moscow, Russia 119991

acting on PSI or PSII, synthetic auxins, was introduced in the early 1950s. Traditional PSII inhibitors such as Dinoseb, diuron, and triazin derivatives reduce electron flow from water to NADPH at the photochemical step in photosynthesis. They bind to the QB site on the D1 protein and prevent quinone from binding to this site. Therefore, this group of compounds causes the electrons to accumulate on chlorophyll molecules. As a consequence, oxidation reactions in excess of those normally tolerated by the cell occur, and the plant dies (Klimov et al. 1989; Allakhverdiev et al. 1989; Trebst and Draber 1979; Govindjee and Eaton-Rye 1986). Some weed species have evolved resistance to the herbicides all over the world. However, no major new site-of-action herbicide has been introduced into the market for about 20 years. Thus, it is very important to find more selective and/or effective chemical compounds, maybe, working through different mechanisms (Eckes et al. 2004).

One of the ways to increase the herbicide efficiency is to design universal compounds, which suppress simultaneously several key enzymes and even enzyme systems. Obviously, PSII is one of such systems, determining the growth and productivity of plant. Another important target of inhibitors action is the plant carbonic anhydrases (CA-s). Recently, the data about several CA classes in higher plants (for example, *Arabidopsis* has three classes of CA-s, it was revealed 19 genes) and about its important role in many biochemical processes determining plant productivity (CO_2 transfer in tissues and cells, its storing and assimilation) were obtained (Moroney et al. 2011). Earlier it was convincingly shown that novel CA activity was also associated with PSII (Stemler 1986; Karlsson et al. 1998; Moskvin et al. 2004; Shutova et al. 2008; Rudenko et al. 2007; Shitov et al. 2009). Furthermore, PSII is one of the main sites of the reactive oxygen forms (ROS) generation in cells (Pospišil 2009). There is a range of enzyme systems against ROS in plants, including glutathione.

Glutathione has a number of functions in biosynthetic pathways: detoxification, antioxidant biochemistry, and redox homeostasis; and these functions are associated with cell cycle, plant development, and cell death (Vivancos et al. 2010; Noctor et al. 2002, 2012). Glutathione exists in two different forms, i.e., the reduced form (GSH) and the oxidized form (glutathione disulfide, GSSG), and physiological functions of glutathione have been mainly attributed to its reduced form in plants (Alscher 1989). GSH is oxidized to GSSG, acting as a major antioxidant in plant cells. Therefore, maintaining high GSH/GSSG ratio in cells is critical (Meister and Anderson 1983). Plants maintain a high proportion of the reduced glutathione by two mechanisms. One mechanism is that GSH can be synthesized in the chloroplast and the cytosol in plant cells. Another one is that GSSG can be reduced to GSH by glutathione reductase (Noctor and Foyer 1998; Asada 1999). Glutathione reductase is a major cellular antioxidant enzyme that is widely distributed both in eukaryotes and prokaryotes catalyzing the reduction of oxidized GSSG to the reduced GSH using NADPH as an electron donor. It has been reported that GR up-regulates under stresses such as salinity, drought, high light intensity, mechanical wounding, chilling, air pollutants, and herbicides.

In our previous work (Karacan et al. 2012, 2014), new organic compounds and their complexes with metal atom, capable of effectively suppressing the photosynthetic electron transfer (PET) and carbonic anhydrase activity (CAA) of PSII, alpha-CA, as well as beta-CA were obtained. It should be noted that alpha-CA from bovine erythrocytes has a structure and activity properties, which are close to those of plant alpha-CA-s. In the work, we revealed the influence of separate side substituents of the agents on its inhibitory efficiency. Obtained information was helpful for design and synthesis of more efficient agents.

In the literature, Zhang et al. have reported that Sb decreases O_2 evolution and fluorescence yield, and damages cellular compounds; in addition, the site of Sb inhibition was on both PSII donor side and acceptor side. Sb exposure resulted in an inhibition of electron transport from QA^- to QB/QB^- and accumulation of $P680^+$ (Zhang et al. 2010). In addition, we know that antimony(III) complexes inhibit the glutathione reductase activity (Wyllie and Fairlamb 2006). In the light of such knowledge, the aim of this work was primary screening of relatively big group of new antimony(III) complexes on the plant photosynthetic and some enzyme activities. Thus, we obtained a series of new antimony(III) complexes, six of them were carboxamide antimony(III) complexes synthesized for the first time. Their structures were identified by elemental analyses, 1H-NMR, 13C-NMR, FTIR, LCMS, magnetic susceptibility, and conductivity measurement techniques. Their most-stable 3D forms were determined by DFT/B3LYP/LANL2DZ calculation method. Their effect on photosynthetic electron transfer and carbonic anhydrase activity of PSII, and glutathione reductase activity of chloroplast was investigated. The influence of side substituents on its inhibitory efficiency was also determined.

Materials and methods

Reagents and solutions

Highly purified GR from baker's yeast (*Saccharomyces cerevisiae*; type IV, Baker's yeast, E.C 1.6.4.2; 160 U per mg^{-1} of protein) and other chemicals (GSSG, GSH,

NADPH, NaH_2PO_4, Na_2HPO_4, and DMSO) were purchased from Sigma-Aldrich (USA). One unit of this commercial preparation reduces 1 μmol GSSG per min at pH 7.2 and 25 °C. In the work, α-carbonic anhydrase isozyme II from bovine erythrocytes (lyophilized powder, 2500 W-A units per mg protein) (C2522, Sigma) was used. All chemicals were of HPLC reagent grade and used without additional purification. All solutions were prepared with ultrapure water. Glutathione reductase stock solution was prepared by dissolving 100 units of GR in 10 mL of phosphate buffer (0.2 M, pH 7.2) and stored at 4 °C. Stock solution of Sb(III) complexes were freshly prepared by dissolving in minimum amount of DMSO and diluting with water to a volume of 2 mL. Other chemicals were reagent grade quality.

Synthesis of the compounds

Six new carboxamide ligands and their antimony(III) complexes (**1**–**6**) were synthesized for the first time substantially in accordance with the procedures as described in our previous study (Aslan et al. 2011; Ozbek et al. 2009; Balaban et al. 2008; Ozmen and Olgun 2008; Ozdemir et al. 2009, 2010; Alcona et al. 2001; Disli et al. 2013). Synthesis of Bis-(5-chloro-*N*-(2-pyrimidine)-2-thiophencarboxamidetrichloroantimony(III) (**1**) is presented below as example.

2-Aminopyrimidine (0.23 g, 2.46 mol) and 5-chlorothiophene-2-carboxylic acid (0.40 g, 2.46 mol) were dissolved in methanol (15 mL) and refluxed for 72 h at 60 °C under stirring. After cooling, colorless product are precipitated, separated, and washed with methanol. Purity was checked by TLC. (m.p. 125 °C, yield 81 %). Anal.Calc. for $C_9H_6ClN_3OS$: IR (KBr, ν/cm^{-1}): 3352.85, 2855.29, 1684.42, 1647.58, and 1583.60. Liquid Chromatography Mass Spectrometry (LC–MS): m/z = 239.22 (M +): 1H-NMR (300 MHz, DMSO-d6): δ 8.30 (1H,–NH) 7.60 (2H, -CH) and 7.25 (1H, –CH) 6.58 (2H, –CH). 13C NMR (100 MHz, DMSO-d6): δ 163.95, 162.37, 158.44, 135.24, 133.55, 134.04, 128.89, and 110.54.

For the preparation of complex (1), a solution of antimony(III) chloride (0.19 g, 0.85 mol) in 10 mL methanol was added slowly to a vigorously stirred solution of 5-chloro-2-thiophenecarboxamide synthesized above (0.30 g, 2.55 mol) in 10 mL methanol. Reaction mixture refluxed for 72 h at 60 °C. After cooling, pale yellow crystals were obtained by slowly evaporation (yield 71 %). $C_{18}H_{12}C_{l5}N_6O_2S_2Sb$: IR (KBr, ν/$cm^{-1}$): 3373, 2861, 1667, 1627, and 1536. Liquid Chromatography Mass Spectrometry (LC–MS): m/z = 708.08 (M+H) + : 1H NMR (300 MHz, DMSO-d6): δ 8.58 (1H, –NH), 8.30 (1H, Hpym), 7.60 (1H, H_{pym}), 7.25 (1H, H_{pym}), and 6.80 (2H, H_{tio}). 13C NMR (100 MHz, DMSO-d_6): δ 162.28, 157.67, 157.18, 135.29, 133.88, 133.83, 128.11, and 110.32.

These six new complexes are (Bis-(5-chloro-*N*-(2-pyrimidine)-2-thiophencarboxamidetrichloroantimony(III) (**1**), *N*-2-pyrimidine-2-piperidinecarboxamidetrichloroantimony(III) (**2**), *N*,*N*-(1,2-phenyl)-2-dipyrrolinecarboxamidetribromoantimony(III) (**3**), *N*-2-pyrimidine-2-pyrolincarboxamidetrichloroantimony(III) (**4**), 2-amino-5-(1H-tetrazole-5-ylthio)-4,6-dimethoxypyrimidine-2-pyrroline carboxamidetrichloroantimony(III) (**5**), *N*-2-benzothiazole-2-pyrrolidinecarboxamidetribromo antimony(III) (**6**).

Compounds (**7–19**) were synthesized, identified, and reported in our previous studies (Tunc et al. 2015a, b).

Structures of all complexes are given in Fig. 1.

Characterization

These compounds were characterized by melting points, elemental analysis, MS, IR, and NMR spectroscopy, and the data were found to be in agreement with those of the assigned molecular structures. The NMR spectra were recorded in d_6-DMSO in a Bruker Ultrashield 300 MHz spectrometer. Elemental analyses were determined on a LECO CHNS-932 auto elemental analysis apparatus. The molecular conductivities of the complexes were measured with WTW Cond 330i. Infrared spectra were obtained using a Mattson 1000 FTIR Spectrometer, from 4000 to 400 cm^{-1} in KBr pellet. Liquid chromatography mass spectra were obtained using a Platform LCMS with methanol–acetonitrile mixture as the solvent. All voltammetric determinations were performed on a CHI 760d potentiostat.

Computational method

Full geometry optimization of all structures was done in gas phase with DFT/B3LYP method using the LANL2DZ basis set implemented in Gaussian 03 (Frisch et al. 2003). The vibrational frequency calculations were performed to ensure that the optimized geometries represent the local minima and that there are only positive eigenvalues. All calculations were performed with the GAUSSIAN03 program package.

Isolation of PSII preparations

Photochemically active thylakoid membrane fragments enriched with PSII (PSII membrane preparations) were prepared from leaves of pea plants (*Pisum sativum* grown for 2–3 weeks) by the method described earlier (Schiller and Dau 2000). These PSII preparations contained about 250 molecules of chlorophyll (Chl) per reaction center (RC) (Klimov et al. 1982) and exhibited O_2 evolution rates of 400–500 μmol mg^{-1} of Chl h^{-1} under saturating light in

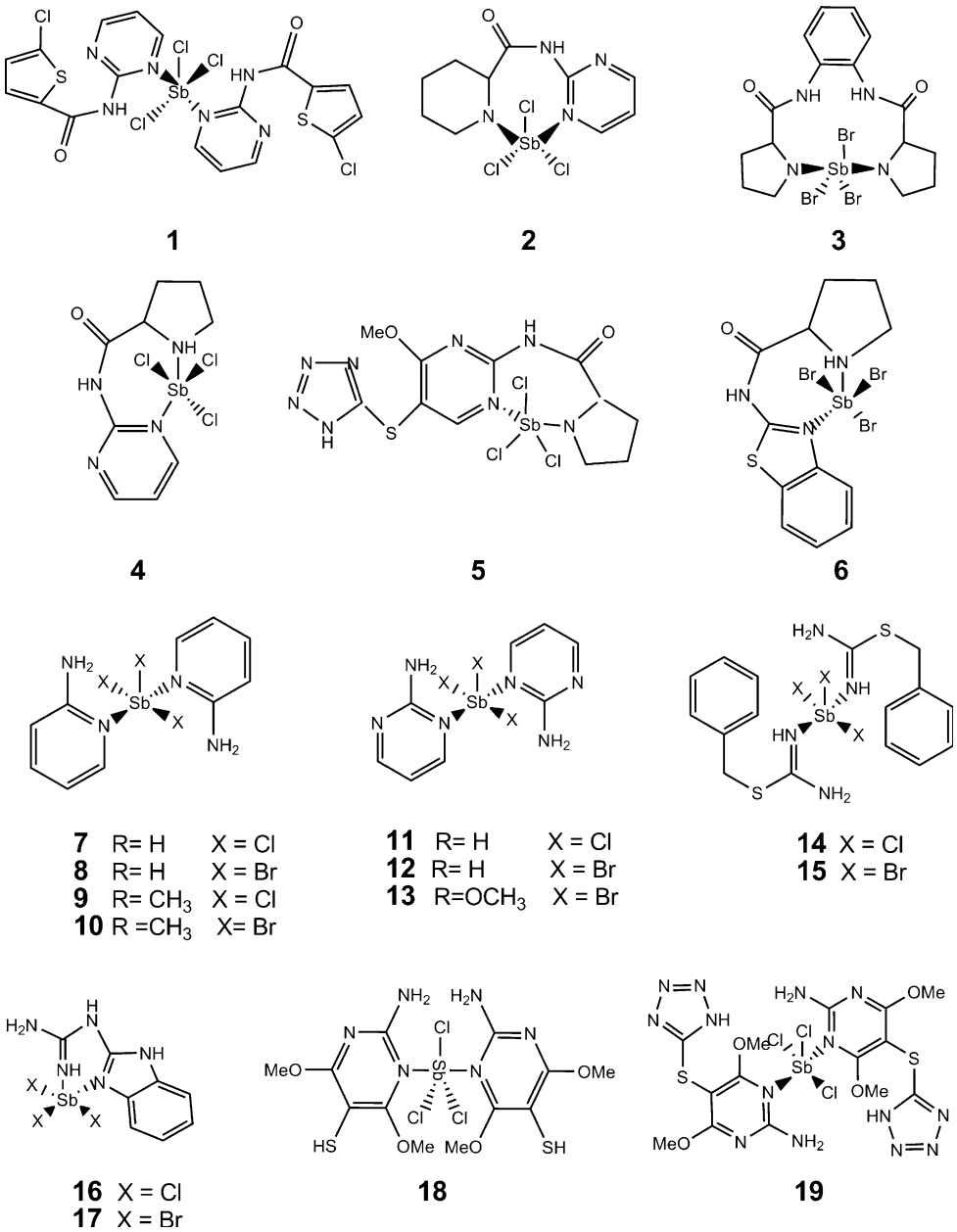

Fig. 1 Structures of the complexes (**1–19**)

the presence of 0.1 mM 2,5-dichloro-pbenzoquinone plus 1 mM $K_3Fe(CN)_6$ as electron acceptors. Samples were stored in liquid nitrogen until use.

Carbonic anhydrase activity measurements

Carbonic anhydrase activity was measured by the electrometric procedure of Wilbur and Anderson (Wilbur and Anderson 1948) as the rate of pH change in CO_2 hydration using Mettler Toledo InLab 413 pH electrode and cpX-2 pH/ion meter (Institute of Biological Instruments (IBI) of the Russian Academy of Sciences, Pushchino) interfaced with a computer. Measurements were carried out at 1.5–2 °C in the medium containing 25 mM veronal (pH 8.6), 50 mM KCl, and 15 mM $MgCl_2$. First, the sample (0.21 mL) was added to 1.29 mL of veronal buffer (the sample addition decreased pH by 0.1–0.15 unit), and then water (0.75 mL, saturated by bubbling with CO_2 at 0 °C for 1 h) was added to the reaction mixture. The time, which was required to decrease pH from 8.3–7.8, was calculated from data of pH change recorded by a special PC program "pX-meter." To express the value of CA activity, Wilbur and Andersen units calculated per mg chlorophyll were used. The calculations were performed according to the formula: $(t_0-t)/(t \times m)$, where t_0 and t are times of changes in pH from 8.3 to 7.8 in the control and in the sample, respectively; m is the amount of chlorophyll in milligrams added to the reaction mixture. The

measurements were carried out 3–4 times using several biological replications.

To analyze the effect of inhibitors on CA activity in the PSII preparations, an inhibitor (at final concentration 100 μM) was added and incubated in the cell for 1 min before beginning the reaction, and then the measuring of reaction was performed.

Chlorophyll a fluorescence measurements

The photochemical activity of the PSII was analyzed by measuring the kinetics of photoinduced changes of the PSII chlorophyll fluorescence yield (ΔF), related to photoreduction of the PSII primary electron acceptor, plastoquinone Q_A, with a pulse-amplitude modulation (PAM) fluorimeter (XE-PAM, Heinz Walz, Germany) and accompanying software Power Graph Professional 3.3 in a 5-mM cuvette at 20 °C. Measurements were carried out in a medium containing 25 mM Mes-NaOH (pH 6.5), 10 mM NaCl. The characteristic values F_v, F_0, F_m, and the maximum quantum photochemical yield of PSII (ratio F_v/F_m), as well as the ratio F_v/F_0 were determined. Here, F_0 is the level of fluorescence before actinic light is applied measured under weak measuring light illumination, and F_m are the maximum fluorescence level, $F_v = F_m - F_0$. To record the F_0 level of fluorescence, the preliminary dark-adapted sample was illuminated by a weak probe pulses of measuring light ($\lambda = 490$ nm; 4 μmol m^{-2} s^{-1}; Xenon-Measuring Flash Lamp, 64 Hz, BG39, Schott). For registration of the photoinduced changes of the PSII chlorophyll fluorescence yield (F_v), the dark-adapted samples were illuminated by the light of saturating intensity (1000 μmol m^{-2} s^{-1}).

Determination of chlorophyll concentration

Total chlorophyll content in the preparations was determined by the method of Arnon (1949) using 96 % (v/v) ethanol.

Isolation of GR enzyme

Isolation of GR from spinach was performed according to literature (Rao et al. 1995).

Glutathione reductase inhibition assay of the chloroplast

Glutathione reductase activity of the chloroplast was determined as described in Kaplan (Kaplan 1985). Glutathione reductase inhibitory activities of the compounds were determined as follows: 25 μM GSSG, 25 μM NADPH, 0.022 unit GR enzyme, and inhibitor solutions added to 10, 25, and 50 nM; 0.5, 2.5, and 5 μM, respectively, were put in 10 mL of dark volumetric flask and incubated 30 min. Decreasing of fluorescence peak of GSH at 30, 60, 120, 180, 240, and 300 s was measured, respectively. Fluorescence values in the inhibition studies were obtained by Hitachi F-7000 spectrometer. There was the maximum decreasing at the 60 s; therefore these fluorescence values and inhibitor concentrations were graphed. Inhibitor concentrations at the half of fluorescence intensity were determined as IC_{50} values.

Glutathione reductase inhibition assay of glutathione reductase from baker's yeast

GR activity was determined according to the Carlberg and Mannervik's method (Carlberg and Mannervik 1985). Glutathione reductase activity was expressed as μmole NADPH oxidized per minute per mg protein, at pH 7.2 at 25 °C, using a molar extinction coefficient at 340 nm of 6.2 $mM^{-1}cm^{-1}$ for NADPH. Decrease in the absorbance at 340 nm due to oxidation of NADPH was monitored with a photodiode array Agilent 8453 spectrophotometer (USA) accompanied UV–Visible ChemStation software. The standard GR reaction medium contained 0.2 M potassium phosphate, 2 mM EDTA (pH 7.2), yeast GR (0.9 units/mL), NADPH (0.2 mM), and GSSG (1 mM). The final volume of the assay mixture was 2.6 mL. The reaction mixture was carefully mixed, and after a 3 min preincubation at 25 °C, the reaction was started by the addition of NADPH, and time-dependent changes in absorbance at 340 nm were monitored. GR activities were measured for 40 s in the quartz cuvette with a 1-cm light path. The reaction was linear during this time period. The initial velocity of GR reaction was measured by the slope of recorded tracing. Not less than seven measurements were performed and mean values were used for each data point. Control represents the activity of GR measured without addition of our complexes, but in the presence of solvent (DMSO) used to dilute the compounds. GR activity of control was equal to about 18 μmol NADPH per min per mg protein. The enzyme activities in the absence of investigated chemicals were taken as 100 %.

Electrochemical properties of the complexes

All voltammetric parameters were measured at room temperature and in aqueous media. Phosphate buffer (0.02 M, pH 7.2) was used as a supporting electrolyte. The number of electrons transferred was found from the chronoamperometric Cottrell slopes of the 1 mM Sb(III) compounds with 1 mM ferrocene as the standard (a reversible transfer of 1 electron) on a C ultramicro disk electrode. Baransky equations were used to calculate the number of electrons transferred (Baranski et al. 1985).

Results

Structure of the compounds

In this study, six carboxamide ligands and their antimony(III) complexes were synthesized for the first time. We could not obtain their single crystals adequate for a crystallographic study, so their structure were characterized using elemental analyses, conductometric measurements, LC–MS, FTIR, and ^{1}H-NMR techniques. LC–MS, ^{1}H-NMR, and ^{13}C-NMR spectra of complexes (**1**–**6**) are given in Figs. S1–S12 (Supplementary Materials).

The molar conductivity data in DMSO solution shows that all complexes are non-electrolyte, that is, they have non charged nature. In the mass spectra of the all complexes, molecular ion peaks corresponding to the $(M+H)^{+}$ species conformed that all complexes were mononuclear structures; their molecular formula were determined as SbL_2Cl_3 for (**1**), and as $SbLCl_3$ for (**2**–**6**).

In the NMR spectra, generally, proton peaks of the ligands were shifted to downfield with complex formations. For example, NH proton peak of aliphatic ring of carboxamide ligands appearing as singlet about 1.70 ppm was shifted to downfield about 2.25 ppm with complexation in the **2**, **4**, **5**, and **6**. Aromatic proton peaks of benzothiazole ring (7.00–7.65 ppm) and aromatic proton peaks (6.35–6.53 ppm) were also shifted to (7.20–7.80 ppm) and (6.80–7.00 ppm) in the **3** and **6,** respectively. Aliphatic proton peaks (1.75–4.40 ppm) also shifts to (1.90–4.40 ppm) with complexation; however, carboxamide NH proton peaks at 8.60 ppm of ligands did not show reasonable shifts with complexation. These results show that carboxamide N-donor atoms do not coordinated to Sb(III) atom. On the other hand, in the IR spectra of the complexes, NH stretching vibrations of pyrroline and pyrimidine groups were shifted to lower frequencies, and the ring stretching vibrations of pyrimidine groups were shifted to higher wavenumber with complex formation. This results showed that bidentate carboxamide ligands coordinate to Sb(III) atom via N-donor atom of aliphatic ring and N-donor atoms on pyrimidine ring.

The geometries of all the complexes were optimized using B3LYP/LANL2DZ method to find most-stable structures. The most-stable structures of (**1**–**6**) are presented in Fig. 2. All complexes have square-pyramidal geometry in the ground state. The equatorial plane in the SP units of **1** is formed by two N, two Cl atoms in trans arrangement and one Cl atom in apical position. 5-chloro-*N*-(2-pyrimidine)-2-thiophencarboxamide in the **1** behaves as monodentate ligand with N-donor atom of the pyrimidine. Similarly, equatorial plane of 3 is formed by two Br, and two N atoms of bidentate *N*,*N*-(1,2-phenyl)-2-dipyrrolinecarboxamide in trans arrangement. On the other hand, N-donor atom of pyrrolidine ring is in apical position in the **2**–**6**; however, N-donor atom of piperidine ring is in the equatorial plane.

Effects of the complexes on photosynthetic electron transfer of PSII

Effects of each from these antimony(III) complexes on the PSII photosynthetic activity were investigated. To estimate the PSII photosynthetic activity and its changes by antimony(III) complexes, we used very informative method—measurements of the photoinduced change of chlorophyll fluorescence yield related to photoreduction of the primary electron acceptor of PSII, plastoquinone Q_A (Klimov et al. 1982). Ratio F_v/F_m, characterizing the PSII maximum quantum photochemical yield of the dark-adapted samples was measured to analyze changes of PSII photosynthetic activity. Complexes and the PSII preparation were preincubated together for 1 min at temperature of 20 °C and stirring prior to assay, in order to allow for the formation of the PSII-Inhibitor complex.

Figure 3 shows typical effects of one from the investigated complexes (compound **18**) on the F_0, F_m, and kinetics of light-induced changes in chlorophyll fluorescence yield (F_v) of subchloroplast membranes enriched in PSII (BBY particles). Compound **18** reduced F_m level (which is equal to $F_0 + F_v$) by suppressing the light-induced changes in fluorescence yield F_v, without affecting F_0 level and the rate of F_v relaxation in the dark (which reflects Q_A reoxidation) (Fig. 3, curve 2). We show that similar effects are characteristic practically for all investigated complexes. These complexes induce a decrease of F_v, while the F_0 level does not change. The rate of dark decay of F_v, reflecting reoxidation of the reduced Q_A, also does not change.

Decrease of the maximum quantum photochemical yield of PSII (ratio F_v/F_m) may be observed in next cases: (a) when F_m declines but F_0 does not change (F_v decreases) (b) when F_0 increases but F_m does not change (F_v decreases) and (c) when both F_0 increases and F_m declines (F_v decreases). Case (a) as usual reflects impairing of the electron donation from the PSII water-oxidizing complex (WOC) to components of reaction center or acceleration of the electron efflux from the PSII acceptor side for example as in the presence of exogenic electron acceptors. Case (b) is characteristic for electron transfer disturbance on the PSII acceptor side for example induced by diuron, known inhibitor of reoxidation of the PSII primary electron acceptor, plastoquinone Q_A. Our complexes induce diminution of the ratio F_v/F_m at the expense of suppression of F_v value. Such reduced F_v is usually observed in this

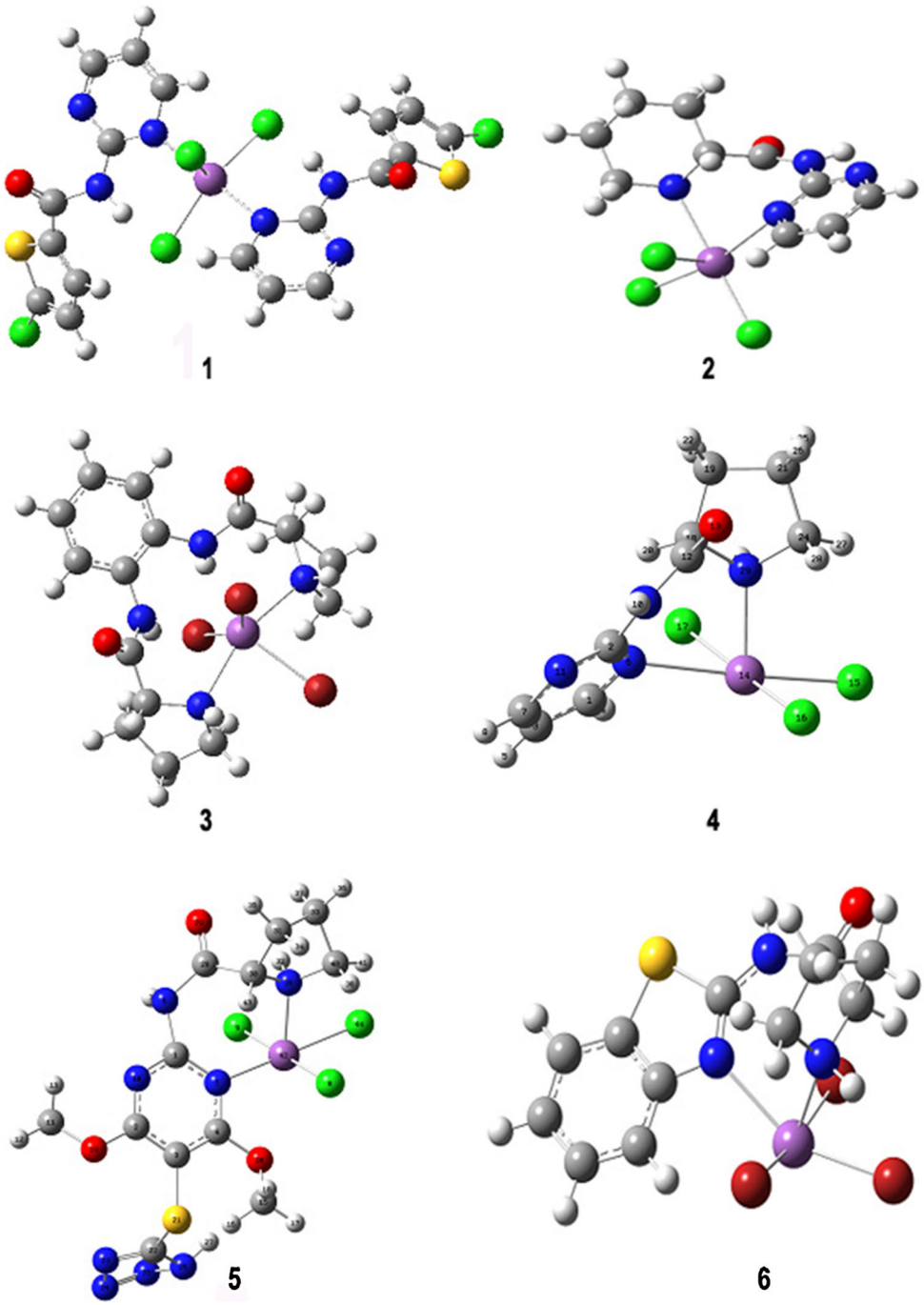

Fig. 2 Optimized structures of the complexes (**14**–**19**)

case, when electron donor capacity of the WOC is limited. Similar effects are typical, when the PSII water oxidation and O_2 evolution reactions are completely inhibited upon depletion of Mn from the WOC. In this case, the F_v may be restored by the addition of the exogenic electron donors. We checked influence of exogenic electron donors on the inhibitory effects of our antimony(III) complexes. Figure 3 shows that restoration of F_v can be achieved by addition of exogenous electron donor: 1 mM sodium ascorbate (curve 3); 150 μM tetramethyl-*p*-phenylenediamine, TMPD (curve 4); and 1 mM potassium ferrocyanide (curve 5). This indicates that our complexes suppress the electron transport on the donor side of PSII.

It should be noted that addition of the indicated exogenic electron donors to the PSII-samples in the absence of the investigated complexes (e.g., **18**) does not change the values F_0 and F_m level (not shown). These chemicals and exogenic electron donors were added as solutions with pH

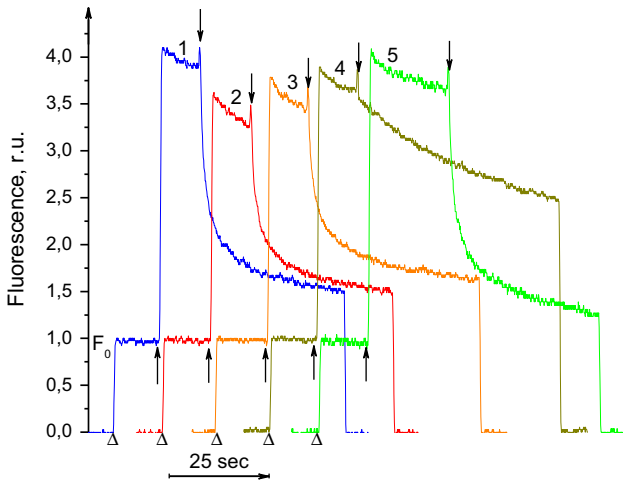

Fig. 3 Kinetics of the photoinduced changes of chlorophyll fluorescence yield (*triangle* F) with excitation by $\lambda > 660$ nm (related to photoreduction of the PSII primary electron acceptor, plastoquinone Q_A) measured in PSII subchloroplast membrane particles in the absence of other additions (**1**) and in the presence of: 0.1 mM compound **18** (**2**–**5**); in the absence of other additions (**2**) and in the presence of 1 mM sodium ascorbate (**3**); 150 μM tetramethyl-*p*-phenylenediamine, TMPD (**4**); 1 mM potassium ferrocyanide (**5**). *Symbol triangle* shows the moment of switching on the measuring light ($\lambda = 490$ nm, 4 μmol photons m^{-2} s^{-1}) which excites PSII chlorophyll fluorescence, F_0 ($\lambda \geq 650$ nm). The *upward* and *downward arrows* indicate the moment of switching on and off, respectively, of the actinic light ($\lambda > 600$ nm, 1000 μmol photons m^{-2} s^{-1}). Chlorophyll concentration was 10 μg mL^{-1}. Each trace is at least an average of seven separate measurements

equal to that of the used buffer. The effects of the electron donors leading to reducing of the complex **18** inhibitory action may be result of direct interaction of these redox agents with the **18** complex. To check this, we investigated the influence each of the electron donors on the absorption spectrum of **18**-agent. We did reveal no changes in the **18**-agent absorption spectrum in the presence of these electron donors (not shown). All similar experiments were done with each from investigated complexes (**1**–**19**).

Inhibition (%) of photosynthetic activity of PSII was calculated according to formula: (F_v/F_m(control)-F_v/F_m(inhibitor)/F_v/F_m(control)] × 100 %. Here F_v/F_m(control) and F_v/F_m(inhibitor) are the values of F_v/F_m in the absence and in the presence of antimony(III) complexes, correspondently. The obtained data about inhibition (%) of photosynthetic activity of PSII of our Sb(III) complexes in concentration of 0.1 mM are given in Table 1 (column 3).

The data presented indicate that practically all complexes show poor efficiency in inhibition of the PSII photochemical activity. Maximal suppression of the ratio F_v/F_m is revealed for complexes **7** (5.0) and **18** (7.0). As it was indicated upper, our complexes do not change the F_0 level. In this case and to reveal effects more obviously, we presented another ratio—F_v/F_0 (Table 1, column 2). The changes of the ratio more sophisticatedly reflect changes of the PSII photochemical activity. In this case, inhibition (%) of photosynthetic activity of PSII was calculated according to similar formula: (F_v/F_0(control)-F_v/F_0(inhibitor)/F_v/F_0-(control)] ×100 %. Antimony(III) complexes **18** and complexes having pyridine, and pyrimidine rings (**7**–**13**) **7**, **19**, and **9** showed best activities because suppress the F_v/F_0 ratio in the range of 10–22 %, on 21.7, 15.7, 12.5, and 9.8 %, correspondently. Less effective inhibitors are complexes **11**, **13**, **3**, carboxamide complex bearing pyrrolidine ring (**4**), or piperidine ring (**2**), inhibition is in the range of 4–9 %. Poor expressed inhibitory action is characterized for complexes **12**, **8**, **10**, carboxamide antimony(III) complexes (**1**, **5**) and *N*-2-benzothiazole-2-pyrrolidinecarboxamidetribromoantimony(III) **(6),** less than 4 %. The antimony(III) complexes of 2-benzyl-2-thiopseudeourea **14** and **15** had no effect on the F_v/F_0 ratio. Generally, (a) chloroantimony(III) complexes showed higher inhibitory activity than bromoantimony(III) complexes, (b) more N donor atom on aromatic ring increases the activity, and (c) electron donating substituents on aromatic ring increase the inhibitory activity.

Effects of the complexes on carbonic anhydrase of PSII and α-CA

One of the factors testifying about optimal function of PSII is also its carbonic anhydrase (CA) activity (Shutova et al. 2008; Shitov et al. 2009, 2011). Therefore, besides influence on the PSII photosynthetic activity, we analyzed effect of the antimony(III) complexes on the carbonic anhydrase activity (CAA) (reaction of CO_2 hydration) of PSII. Figure 4 shows typical kinetics of the pH changes characterizing carbonic anhydrase activity (reaction of CO_2 hydration) of BBY particles in the absence (curve 2) and in the presence of Sb(III)complexes **18** and **11** (curves 3 and 4, respectively). The CA activity measured in the absence of our complexes was used as control (100 % CA activity) and was equal to 12.0 ± 1.4 Wilbur-Anderson units per mg of chlorophyll or (in other units) to 110 ± 15 μmol H^+ per mg Chl per min. The kinetic of the spontaneous CO_2 hydration occurring in the measuring medium in the absence of PSII without any additions shown in Fig. 4 (curve 1) was used as a negative control. Each trace is an average of at least seven separate measurements. We studied effects of each from the Sb(III) complexes (**1**–**19**) on the CA activity of PSII particles.

Furthermore, similar experiments were done with α-carbonic anhydrase isozyme II from bovine erythrocytes. In this case, final concentration of protein was 0.0005 mg mL^{-1}. Initial carbonic anhydrase activity of protein (without additions) was equal to 2500 Wilbur-Anderson units per 1 mg of protein. We found that in both

Table 1 Effects of antimony(III) complexes (in concentration of 0.1 mM) on the PSII photosynthetic activity

Compounds (0.1 mM)	$[(F_v/F_0(c)-F_v/F_0(i)/ F_v/F_0(c)] \times 100$ %	$[(F_v/F_m(c)-F_v/F_m(i)/ F_v/F_m(c)] \times 100$ %
1	3.2 ± 0.2	1.3 ± 0.08
2	5.7 ± 0.3	2.0 ± 0.11
3	4.2 ± 0.2	1.6 ± 0.08
4	7.0 ± 0.4	2.4 ± 0.14
5	2.5 ± 0.1	1.2 ± 0.05
6	2.2 ± 0.2	1.1 ± 0.10
7	15.7 ± 0.3	5.0 ± 0.10
8	1.9 ± 0.1	1.0 ± 0.05
9	9.8 ± 0.2	3.2 ± 0.7
10	1.7 ± 0.1	1.0 ± 0.06
11	9.0 ± 0.4	3.0 ± 0.13
12	3.5 ± 0.2	1.4 ± 0.08
13	6.7 ± 0.3	2.3 ± 0.10
14	0.0 ± 0.1	0.0 ± 0.10
15	0.0 ± 0.1	0.0 ± 0.10
16	–	–
17	–	–
18	21.7 ± 0.5	7.0 ± 0.16
19	12.5 ± 0.4	4.0 ± 0.13

Inhibition (%) of photosynthetic activity of PSII was calculated as $(F_v/F_m(c)-F_v/F_m(i)/F_v/F_m(c)] \times 100$ % (column 3) or as $(F_v/F_0(c)-F_v/F_0(i)/F_v/F_0(c)] \times 100$ % (column 2). The $F_v/F_m(c)$ or $F_v/F_0(c)$ and $F_v/F_m(i)$ or $F_v/F_0(i)$ are the values of F_v/F_m or F_v/F_0 in the absence (control) and in the presence of antimony(III) complexes, correspondently. Complexes and the PSII preparation were preincubated together for 1 min at temperature of 20 °C and stirring prior to assay, in order to allow for the formation of the PSII-Inhibitor complex. Sign (–) means not measured. Each data is an average of at least seven separate measurements

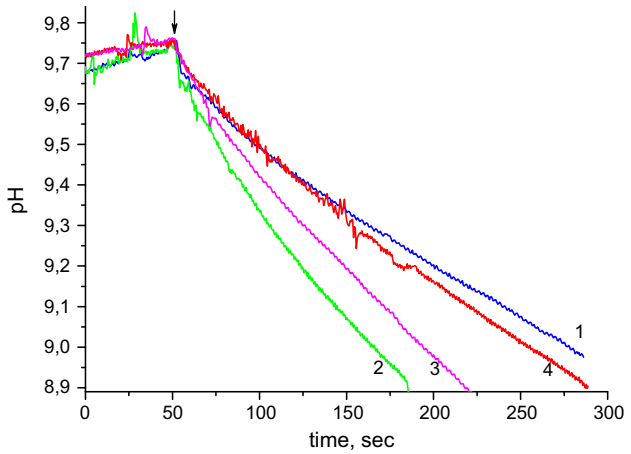

Fig. 4 Kinetics of pH changes characterizing carbonic anhydrase activity (reaction of CO_2 hydration) of PSII (100 μg Chl, final concentration of Chl 44 μg mL^{-1}) in the absence of other additions (**2**), in the presence of 0.1 mM complex **18** (**3**), and complex **11** (**4**). Kinetics of spontaneous reaction of CO_2 hydration occurring in the measuring medium in the absence of PSII without any additions (**1**). *Downward arrow* indicates the moment of addition of CO_2-satured water. Each trace is an average of at least seven separate measurements. 100 % carbonic anhydrase activity of PSII (without additions) was equal to 12 Wilbur-Anderson units per 1 mg Chl

cases with PSII-samples and α-carbonic anhydrase the antimony(III) complexes did not affect the spontaneous reaction of CO_2 hydration. Activity of carbonic anhydrase from PSII in the presence of our antimony(III) complexes is presented in Table 2.

It should to note that almost all complexes decreased CA activity of PSII with relatively potent but different efficiency. About half of them revealed high inhibitory activity (more than 50 % inhibition) and last half—moderate (less 50 %). As shown in Table 2, several complexes exhibit good activity, such as **11**, **18**, **1**, and **14** (above 60 % of inhibition). Among them, complex **11** has maximal efficiency 90.2 %. Bromo antimony(III) complexes having methoxy substitute pyrimidine ring has more activity. Complexes **13**, **8**, **10**, **4**, and **2** have moderate inhibitory action from 41 to 20 %. Generally, antimony(III) complexes exhibited good inhibitory effect on carbonic anhydrase from PSII. Compounds **5**, **12**, **19**, **6**, **3**, and **7** had weak inhibitory efficiency (less 20 %). Complex **15** had no influence on the CA activity of PSII. It should be noted that complex showing good photosynthetic electron transfer inhibitory efficiency (**18**) also exhibits good PSII carbonic anhydrase inhibitory effect, but complexes with

Table 2 Inhibition (%) of CA activity of PSII and of α-CA from bovine erythrocytes by our Sb(III) complexes (**1**–**19**) in concentration of 0.1 mM

Compounds (0.1 mM)	Inhibition of CAA of PSII (%)	Inhibition of CAA of α-CA (%)	
		Incubation time	
		2 min	30 min
1	62.5 ± 2.9	13.8 ± 0.7	18.7 ± 0.8
2	22.9 ± 1.4	13.5 ± 0.6	99.3 ± 4.1
3	9.9 ± 0.6	13.5 ± 0.7	83.9 ± 3.6
4	25.1 ± 1.8	20.0 ± 1.1	31.0 ± 1.4
5	18.4 ± 1.1	0.0 ± 0.1	0.0 ± 0.2
6	11.1 ± 0.7	20.5 ± 1.1	92.3 ± 4.2
7	9.4 ± 0.5	0.8 ± 0.1	49.2 ± 2.5
8	31.6 ± 1.3	8.7 ± 0.3	29.9 ± 1.3
9	60.6 ± 4.2	3.6 ± 0.1	20.0 ± 0.8
10	30.7 ± 1.2	0.7 ± 0.1	64.1 ± 3.8
11	90.2 ± 4.3	5.7 ± 0.3	98.4 ± 3.2
12	16.3 ± 0.6	0.0 ± 0.2	57.6 ± 2.4
13	40.1 ± 2.1	15.0 ± 0.7	53.4 ± 2.1
14	54.4 ± 3.6	23.8 ± 1.0	26.7 ± 1.1
15	–	21.1 ± 1.2	36.0 ± 1.0
16	10.0 ± 0.4	–	–
17	–	–	35.0 ± 1.3
18	64.8 ± 4.0	91.1 ± 3.6	99.3 ± 3.6
19	14.3 ± 0.8	–	–

In both cases, CA activities without additions of any inhibitors were used as control (0 % of inhibition, 100 % activity). In the case of α-CA compound and enzyme, solutions were preincubated together for 2 min or 30 min at room temperature prior to assay, in order to allow for the formation of the C-E complex. Sign (–) means not measured. Each data is an average of at least seven separate measurements

potent inhibitory action on the PSII CA activity such as **9**, **11**, and **14** had weak effect on the PSII photosynthetic activity.

Previously, results indicating the importance of carbonic anhydrase activity for the operation of the PSII were obtained (Shitov et al. 2011). Another carbonic anhydrases located in the cytoplasm and chloroplast stroma (Badger and Price 1994) as well as in thylakoid lumen (Fedorchuk et al. 2014) are also very important for the functioning of plant cells. Therefore, it was important to determine the effect of our complexes on the activity of another carbonic anhydrase of plant cells. In plants Genes of three (α, β and γ) families of carbonic anhydrase were found, many of them are expressed (Moroney et al. 2011). As a model for our research, α-carbonic anhydrase from bovine erythrocytes was selected because its amino acid sequence and structure similar to those of α-carbonic anhydrase from plants. In addition, it was received a lot of data about effects of various inhibitors on animal α-carbonic anhydrase. Therefore, we had an excellent opportunity to compare the inhibitory efficiency of our Sb(III)complexes with that of the known carbonic anhydrase inhibitors. Effects of our antimony(III) complexes on the CA activity of α-carbonic anhydrase from bovine erythrocytes are also presented in Table 2. As rule in the case of α-carbonic anhydrase the inhibitory efficiency of majority from the Sb(III)complexes tested is increased in time-dependent manner (see complexes **2**, **3**, **6**, **7**, **10**, **11**, **12**, **13**, **15**, **18**). However, in the case of complexes **1**, **4**, **14**, the inhibitory action does not increase after 30 min incubation. Complexes **18**, **2**, **11**, and **6** suppressed CA activity of more than 90 %, and their inhibitory effect is comparable to that of the known effective inhibitors of carbonic anhydrase (ethoxyzolamide, acetazolamide, and other (Ignatova et al. 2006; Rudenko et al. 2007; Shitov et al. 2009)). Complexes **7**, **15**, **4**, **8**, **14**, and **9** showed moderate inhibitory effect (in the range 50–20 %). Complex **1** has weak inhibitory effect (less than 20 %). Compounds **5** and **19** did not inhibit CA activity of α-carbonic anhydrase from bovine erythrocytes. It is interesting that some complexes have different inhibitory potency on CA activity of PSII compare to that of α-carbonic anhydrase from bovine erythrocytes, especially after 30 min incubation.

Effects of the complexes on glutathione reductase from spinach chloroplast

IC_{50} values obtained after 5 min of incubation of our Sb(III) complexes with the glutathione reductase enzyme isolated from spinach chloroplast are given in Table 3. In general, all antimony(III)complexes showed good inhibitory effect on glutathione reductase activity at the nanomolar level. *N*-2-benzothiazole-2-pyrrolidinecarboxamidetribromoantimony(III) complexes (**6**) exhibited the best activity, and 2-guanidobenzimidazoletribromoantimony(III) complex (**17**) also showed similar inhibition properties. Complex (**18**) bearing SH substituents on pyrimidine rings behaved as good inhibitor against glutathione reductase from baker's yeast *S. cerevisiae* (9.19 μM) (Tunc et al. 2015a) and glutathione reductase from chloroplast (0.020 μM—Table 3). If we compare these values, we found that glutathione reductase activity is more strongly depressed in plants than in *S. cerevisiae*. Similarly, **19** exhibited more inhibitory activity in plant (0.030 μM) than in *S. cerevisiae* (28.67 μM—Table 3). Among the seven complexes (**7**–**13**), **13** showed the best activity followed by **12**. Generally, (a) bromoantimony(III) complexes have higher activity than chloroantimony(III) complexes, (b) more N-donor atom on aromatic ring increases the activity, and (c) electron donating substituents on aromatic ring increase the inhibitory activity.

Table 3 Glutathione reductase inhibitory activity (IC_{50}) of the Sb(III) complexes

Compound	IC_{50} (μM)
1	0.060
2	0.042
3	0.018
4	0.017
5	0.034
6	0.010
7	0.025
8	0.013
9	0.020
10	0.017
11	0.019
12	0.012
13	0.011
14	0.020
15	0.016
16	0.017
17	0.010
18	0.020
19	0.030

GR is from spinach chloroplast

Effects of the complexes on glutathione reductase from baker's yeast

To test the specificity of action of our Sb(III) complexes (**1**–**19**) on the activity glutathione reductase from various source, we also used a known model object—glutathione reductase from baker's yeast. This allowed us to compare the inhibitory activity on our complexes with known inhibitors of glutathione reductase. Effect of investigated compounds (**1**–**19**) in concentration of 0.1 mM on the activity of glutathione reductase from baker's yeast are presented in Table 4 as a (%) of inhibition. Control is the GR activity measured in the absence of inhibitor (100 % activity).

It should be noted that all Sb(III) complexes more or less inhibited the activity of glutathione reductase from baker's yeast. Table shows that more than half from our complexes inhibit the GR activity by more than 50 %. These are complexes **2**, **14**, **7**, **9**, **11**, **5**, **10**, **13**, **4**, and **19**. Among them, complex **2** inhibits the GR activity by 90 % and complexes **14** and **7**—more than 75 %. Complexes **8**, **3**, **6**, **12**, **1**, and **15** have moderate inhibition efficiency (from 49

Table 4 Effect of investigated compounds (**1**–**19**) in concentration of 0.1 mM on the activity of glutathione reductase from baker's yeast (*S. cerevisiae*)

Compound	Inhibition (%) GR from baker's yeast
Control	0 ± 0.1
1	35.4 ± 1.6
2	90.0 ± 4.0
3	48.5 ± 2.2
4	62.3 ± 2.6
5	68.0 ± 2.8
6	41.1 ± 1.3
7	75.5 ± 3.2
8	48.9 ± 2.1
9	73.6 ± 2.9
10	66.8 ± 2.5
11	68.1 ± 2.4
12	41.1 ± 1.2
13	65.8 ± 2.4
14	77.5 ± 3.3
15	32.0 ± 1.4
16	–
17	–
18	17.0 ± 0.6
19	55.6 ± 2.4

Control is the GR activity measured in the absence of inhibitor (0 % of inhibition, 100 % activity). Sign (–) means not measured. Each data is an average of at least seven separate measurements

to 20 %). Compound **18** showed little inhibitory effect (17 %).

Thus, practically all complexes tested exhibit high inhibitory activity against both glutathione reductases from spinach chloroplasts and from baker's yeast.

Electrochemical properties of the compounds

Cyclic voltammetric analysis of the antimony(III) compounds were performed in a mixture, containing 100 μL compound stock solution and 10 mL phosphate buffer (0.02 M, pH 7.2) at various scan rates (10, 50, 100, 500, 100 mV s − 1) in the potential range between 0.0 and −1.65 V under a dinitrogen atmosphere. Electrochemical data of the compounds are summarized in Table 5. As seen in Table 5, the number of electron transferred is three; cathodic and anodic peaks are asymmetrical (peak separation is large); and ratios of reverse to forward peak currents (ia/ic) were ranging between 1.4 and 2.7. These results indicate that the system is irreversible and correspond to Sb(III)/Sb(0) couple. These findings were corresponded to previous study (Karacan et al. 2015).

If we compare the GR inhibition activity of the complexes (**7**–**13**) with their potential $E_{1/2}$, there is a relationship. The best GR inhibitor **13** has the biggest negative potentials, that is, it reduces more difficult than others. Similar correlation was also observed among the carboxamide antimony(III) complexes (**1**–**6**); the higher the reducing potentials value, the greater the inhibitory activity. Additionally, bromo complexes (**15** and **17**) of the same ligands, 2-guanidobenzimidazole and 2-benzyl-2-thiopseudeourea, have big reducing potential than those of chloro complexes (**14** and **16**), respectively.

Table 5 Some electrochemical data of the antimony(III) compounds

Compounds	Ep_c–Ep_a (mV)	$Ep_{/2}$ (*V*)	ip_a/ip_c	*n*
1	−260	−0.580	1.460	2.89
2	−420	−0.485	1.570	3.07
3	232	−0.343	2.786	2.99
4	−310	−0.336	1.430	3.03
5	−100	−0.481	1.521	3.00
6	240	−0.321	2.764	2.94
7	333	−0.346	1.864	2.68
8	320	−0.336	1.880	2.88
9	−200	−0.344	1.526	2.92
10	106	−0.338	1.044	2.85
11	281	−0.342	1.406	3.06
12	265	−0.326	1.398	3.01
13	−251	−0.304	1.963	3.01
14	266	−0.420	2.825	2.90
15	−119	−0.332	2.106	2.99
16	247	−0.376	3.240	3.03
17	−509	−0.278	1.825	3.11
18	−206	−0.522	1.822	3.10
19	−220	−0.321	1.901	2.94

n number of electron transferred

Discussion

Inhibition of PSII photosynthetic activity

From data on Fig. 1 and in Table 1, it is evidently that complexes containing antimony atom and benzene derivatives with one or two N atoms in benzene rings (**18**, **7**, **19**, and **9**) have better inhibitory effect on the PSII photochemical activity. It should be noted that all from the above complexes antimony atoms are coordinated with chloride ions (Cl^-). These ions are probably important for binding of the inhibitory molecule onto the PSII action site, because similar complexes **8** and **10** (containing Br^- ions instead of Cl^- ion) have much less inhibitory activity. It is possible that these complexes displace chloride ions from their binding sites on the PSII donor side near Mn_4CaO_5-cluster, and thus, can disrupt the structure of the WOC as well as the electron transfer on the donor side of PSII. The results of F_v measurement in the presence of artificial electron donors presented in Fig. 3 confirm this proposition. The fact that the complexes **2** and **4** also have an inhibitory effect, in spite of they have a different structure of the organic moiety also testifies in favor of a major role of chloride ions in the inhibition efficiency. An organic moiety of these complexes apparently also plays certain role in inhibitory activity (see complex **7**). The inhibition efficiency of complexes with brome ion instead of chloride ion is increased if the complex contains two N atoms in benzene ring and OCH_3 group as it is observed in case of the complex **13** (Fig. 1). It is possible that the nature of substituents (side groups) plays a certain role: complex **9** with the CH_3 as side group has somewhat reduced inhibitory activity in comparison with similar complex **7** without the side group. Presence of SH-groups (complex **18**) may also increase the inhibitory capacity. This may be due to the rupture of the disulfide bridges of the PSII proteins resulted from redox reduction as well as a possible mechanical influence on the structure of WOC. Anyway, it is difficult to speak about the exact mechanism of action of these inhibitors on the PSII, and it is evidently that further studies are required.

Inhibition of the PSII carbonic anhydrase activity

It was previously shown that a known sulfanilamide inhibitor of carbonic anhydrase, ethoxyzolamide, was more

effective in inhibiting the PSII carbonic anhydrase activity than another known sulfanilamide carbonic anhydrase inhibitor, acetazolamide (Ignatova et al. 2006; Rudenko et al. 2007; Shitov et al. 2009). Perhaps, it was due to more lipophilicity of ethoxyzolamide. Its greater lipophilicity compared with acetazolamide may be due to the presence of two conjugated cycles in the structure of the molecule (Lewis et al. 1984), while in the molecule of acetazolamide only one cycle is present (Granero et al. 2008). In case of Sb(III) complexes the data obtained did not confirm the assumption that the inhibitory effect on the PSII carbonic anhydrase activity directly depends on the number of conjugated cycles. It was found that complexes containing a heterocycles which does not conjugated with other cycles (**11**, **18**, **1**, and **14**) are capable to more effectively suppress the carbonic anhydrase activity of PSII.

Efficiency of the activity suppression by complex **11** is close to that of ethoxyzolamide on the contrary to complexes containing two–three conjugated cycles (complexes **4**, **2**, **5**, and **6**). However, we can not exclude that inhibitory properties are less dependent on the presence/absence of several conjugated heterocycles, and the presence of specific groups in the molecule of the inhibitor is more important for inhibition efficiency. For example, complexes **11**, **18**, **1**, and **14** according their structure do not be attribute to sulfonamides (they do not contain a sulfanyl group) but, nevertheless, they contain amide groups. Apparently the efficiency of suppression of the PSII carbonic anhydrase activity is associated with the presence of these groups. In addition, in complexes **11**, **18**, **1**, and **14,** antimony(III) ion is a ligand for chloride ions. It is possible that these ions are involved in the attaching of the above complexes onto WOC. The presence of Br^- ions instead of Cl^- in complexes **12** and **15** (which are similar to complexes with high inhibitory activity, **11** and **14**, respectively) decreased the inhibitory effect in case of complex **12** or led to a complete loss of inhibitory potency in case of complex **15**. This property of Cl^- ions to enhance the inhibitory effect on the PSII carbonic anhydrase activity is not typical for inhibition of α-carbonic anhydrase from erythrocytes (Table 2). Among the most potent inhibitors of the α-carbonic anhydrase there are complexes containing with Br^- (complex **6**), although the greatest amount of inhibitors still contain chloride ion as antimony ligand. It should be noted that complexes 18 and 11 are both potent inhibitors of carbonic anhydrase and photosynthetic activity of PSII (Tables 1, 2). This inhibitory effect is related to the donor side of PSII (at least for complex **18**), as it was shown in experiments with artificial electron donors (Fig. 3). These results indicate the presence of the relationship between carbonic anhydrase and photosynthetic activity and the importance of carbonic anhydrase activity for the functioning of the donor side of PSII. These data are close to the results obtained at the same samples (BBY particles) using the sulfonamide inhibitor, acetazolamide (Shitov et al. 2011). However, to fully confirm the hypothesis about the importance of carbonic anhydrase activity for the operation of the PSII, additional experiments with Sb(III)complexes, as well as with another carbonic anhydrase inhibitors are need.

Inhibition of carbonic anhydrase activity of α-CA

Table 2 shows that the inhibitory effect of almost all Sb(III)complexes on α-carbonic anhydrase from erythrocytes has time-dependent manner at room temperature. Unfortunately, the PSII photosynthetic activity is starting to decrease at room temperature during this time region. Therefore, a study of the dependence of the PSII carbonic anhydrase activity upon the incubation time was inappropriate. Incubation for 30 min of α-carbonic anhydrase with Sb(III)complexes reveals that this period almost a third of them exhibits high inhibitory activity, whereas half of these complexes suppressed the activity of α-CA almost completely (**18**, **2**, **11**, and **6**). It should be noted that complexes **18** and **11** inhibited also both the photosynthetic and CA activity of PSII (Tables 1, 2). The complex **2**, effectively inhibiting the activity of α-CA, has an average capacity on photosynthetic and CA activity of PSII. Complex **6** had a slightly pronounced inhibitory effect on the activity of PSII, while having the ability to significantly inhibit CA activity of α-carbonic anhydrase. Perhaps little effect on the PSII activity of complexes **2** and **6** is determined by the molecule structure of these compounds, which contain 3-4 cycles and are relatively large to pass inside WOC. Apparently, the nature of the ligand atom for antimony (Cl^- or Br^-) is irrelevant to the effectiveness of inhibitory effect on α-carbonic anhydrase. It is possible that these substances inhibit α-carbonic anhydrase, not only due to the binding to the Zn^{2+} ion active site (as is typical for the mechanism of action of sulfonamides (Supuran et al. 2003), but also due to another mechanisms (for example, by block of channel for the substrate inflow/protons removal). No doubt, further studies on the mechanism of action of these inhibitors are need. This question is very interesting and important from a practical point of view, as it will allow developing new medicines. Complexes **5** and **19** did not inhibit α-carbonic anhydrase (Table 2). Perhaps this is due to the rather large size of the complex (Fig. 1), which prevents the penetration to the enzyme active center.

Inhibition of glutathione reductase from spinach chloroplasts

From Table 3 and Fig. 1, it can be seen that complexes (**8**–**10** and **11**–**13**) with the similar molecule structure had the

greatest inhibitory effect. Moreover, the presence in the molecule heterocycle second N atom apparently resulted in a stronger inhibitory effect. It should be noted that all of the most active agents have in the composition of the complex bromine ions bonded with antimony ion. The inhibitory effect of the similar complexes but with Cl^- instead Br^- decreased (as, for example, in case of complexes **11**, **7**, and **9**). Probably the bromide ion plays an important role in the mechanism of inhibition of the glutathione reductase from chloroplasts. This assumption is supported by the fact that almost 2/3 of highly efficient inhibitors contains Br^- ion. Apparently, the organic part of the molecules of these complexes contributes significantly less to the mechanism of the inhibitory effect, since the inhibitory activity decreases by about 2 times when Br^- is changing to Cl^-. In addition, the inhibitory activity changes little, if complex contains Br^- ions, and organic part of the molecule is modified. For example, in case of the complexes **12** and **13**, its inhibitory activity varies slightly if -OCH3 group is attached to the heterocycle. The fact that even such different in structure complexes as **6**, **13**, and **12** show close-inhibiting ability (Table 3) testifies in favor of this hypothesis.

Inhibition of glutathione reductase from baker's yeast

Several different character inhibitory action of Sb(III)-complexes is observed on glutathione reductase from yeast (Table 4). Complexes containing chlorine ions (**2**, **14**, **7**, **9**, **11**, and **5**) exhibit greatest inhibitory activity. From complexes with bromine ion, only complexes **10** and **13** exhibit quite high inhibitory activity. Other Br^--containing complexes inhibit the activity of not more than 50 %. Apparently, the structure of the complex organic moiety plays an important role in the inhibitory potency of these compounds. The fact that the inhibitory effect of complex **2** higher than that of complex **4** by 30 % testifies in favor of this hypothesis. However, difference in the structure of the organic part of these molecules is only in amount atoms in heterocycle bearing atom N: complex **2** has hexaatomic heterocycle with atom N whereas complex **4** has pentaatomic heterocycle with atom N. Apparently molecular structure inherent for complex **4** and repeating in complex **5** is less optimal for effective inhibition than the structure of complex **2**. At the same time, the complex **5** has greater inhibitor ability as compared with complex **4**. This may be due to the presence in complex **5** additional pentaatomic aromatic heterocycle with 4 atoms N. It is possible that this cycle plays an important role in the inhibitory mechanism, since it is also present in complex **19**, which exhibits a 3-fold greater inhibitory power than complex **18** (in which this functional group is absent). It should be noted that complexes containing motif $-N = C(H)-(NH)$ (e.g., complexes **7–10** and **11–13**) are effective as inhibitors, despite the presence of bromine ion. It is possible that this motif plays an important role in binding of the inhibitor molecule in the enzyme active site. The presence of this motif associated with a sulfur atom (as in complexes **14**, **5**, and **16**), possibly enhances bind of inhibitor with enzyme. However, to confirm this hypothesis, further researches are needed. Compounds based on the organic part as in complexes **7–10**, **11–13**, and **14–15** are the most effective and promising for further study and implementation, because they effectively inhibit glutathione reductase activity of both chloroplast enzyme and yeast one. The use of these organic compounds and their derivatives as herbicides enable inhibit glutathione reductase in chloroplasts and cytoplasm of green parts of the weeds. Treatment plants with these substances can lead to more rapid destruction of weeds than by currently used herbicides.

Conclusions

Nineteen antimony(III) complexes (among them six carboxamide antimony(III) complexes were synthesized for the first time) were obtained and their structure were identified with common methods. The most-stable structures were calculated with DFT/B3LYP/LanL2DZ method. In general, almost all antimony(III) complexes showed inhibitory effect on photosynthetic activity of PSII (at the micromolar range), as well as glutathione reductase activity (at the nanomolar level). In addition, some of them inhibited carbonic anhydrase activity of PSII. For example, **11** influences three active sites: suppresses the photosynthetic activity, carbonic anhydrase activity of PSII by 90.2 %, and glutathione reductase activity significantly by (0.019 μM). Generally, bromoantimony(III) complexes have higher inhibitory activity on chloroplast glutathione reductase than chloroantimony(III) complexes; reducing potential of the complexes correlates with their glutathione reductase inhibition activity. Decrease in reducing potential would be favorable for the glutathione reductase inhibitory activity. As a matter of fact, antioxidant flavoenzyme glutathione reductase reaction was catalyzed by a single-electron reduction with the subsequent redox cycling, and oxidative stress accelerates with an increasing the quinone/semiquinone redox couple. Philippe Grellier et al. implies that compounds with a low reduction potential may be used as relatively efficient antiplasmodial agents with a low mammalian cell cytotoxicity (Grellier et al. 2011).

Substances which have an organic moiety like that in complexes **7–10** and **11–13** are the most versatile inhibitors both photosynthetic activity and associated with a PSII

carbonic anhydrase activity, as well as glutathione reductase activity of chloroplasts. This property may be used in the design of new, highly efficient herbicides for agriculture. In addition, these compounds effectively inhibit α-carbonic anhydrase and glutathione reductase from backer's yeast. The data obtained about inhibitory activity of Sb(III) complexes may be useful in developments of new drugs and antibiotic (fungicide) agents. Complexes **7–10** and **11–13** are the most promising basis for the studies of the influence of molecular structure on the inhibition efficiency of photosynthetic, carbonic anhydrase, and glutathione reductase activities. Special attention in subsequent studies should be paid to the influence of nitrogen atoms (or groups of atoms N(or S)-C(NH)=N(H) in the heterocycle on inhibitory potency of substances.

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